

# Laboratory Diagnosis of Typhoid Fever

## With Notes on Treatment

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TYPHOID FEVER is so rare in most large cities of the United States, where medical teaching is centered, that many physicians complete their internship and residency training without ever having seen a case of the disease. They are therefore not "typhoid-conscious" and do not consider this possibility in differential diagnosis. At the Los Angeles County General Hospital there are about 15 cases a year. Most of these are incurred abroad, for although the United States Public Health Service advises immunization against many other diseases for citizens leaving the country, typhoid fever immunization is regrettably not included.

Clinical signs and symptoms of typhoid fever have been described many times and are helpful in diagnosis, but the purpose of this presentation is to emphasize that even when these findings are typical, the only measure which establishes the diagnosis is the isolation of the infecting organism from the blood. Usually it is present in the blood during the first week, but experience of 35 years indicates that some degree of bacteremia persists from onset to final defervescence. As the factors of immunity come into play, more particularly in the second week, the reticuloendothelial fixed-tissue phagocytes are stimulated to a maximum, and the spleen and liver (which become perceptibly enlarged) take up large numbers of the bacteria; therefore fewer and fewer are present in the blood. This sequence of events must be taken into consideration in obtaining a positive response to blood culture.

It is unusual for the Widal agglutination test to give a positive response before the second week. In the author's experience the positive response may be delayed until the sixteenth week and in many cases it is never obtained, as this presentation reports.

The following resume of 41 cases of typhoid fever, observed at the Los Angeles County General Hospital between June 1, 1953, and May 15, 1956, exemplifies the diagnostic laboratory methods employed in the communicable disease unit of the hospital.\*

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\*All laboratory procedures are done under the direction of Jean W. Dedrick, Ph.D., director of the communicable disease laboratory.

• As indicated by a study of 41 cases of typhoid fever treated in three years, blood culture alone is often sufficient for the diagnosis of the disease if a large (30 cc.) specimen is used. Demonstration of the organism is the only completely diagnostic measure, but this was also achieved by the Widal reaction, by fecal or urine culture, or by aspirated bile culture, which in one case gave the only positive response.

Chloramphenicol is the drug of choice in treating typhoid fever. Since only 25 per cent of patients develop immunity, immunizing injections should be started a week after therapy is discontinued.

*Blood Culture:* Approximately 10 cc. of blood is added aseptically to 100 cc. of tryptose phosphate broth and a second 10 cc. of blood to 100 cc. of bile broth. If no growth occurs in either medium, a second culture is made of 30 cc. of blood added to 300 cc. of bile broth. If growth of Gram-negative bacilli occurs, the organisms are tested for motility and identified biochemically. Specific antisera are used for final identification; the organisms being tested against Kauffmann-White Group D, flagellar H and Vi antisera. When agglutination is produced, the organism is identified as *S. typhosa*. Since there are far fewer bacteria in the blood after the first week of the disease, the use of larger (30 cc.) blood specimens is logical, and is validated by the high frequency of positive response—29 out of 41 cases, in the present series, a higher proportion of diagnosis than was obtained by any other method.

*Fecal Culture:* Rectal swabs are placed in sterile normal saline solution. About 1 gram of feces is suspended in 5 cc. of saline solution. A loopful of the resulting suspension from each is then streaked on Shigella-Salmonella agar. Nonlactose-fermenting colonies are identified as described under blood culture.

*Urine Culture:* After the second week, should the disease run that long under modern therapy, it is quite common to find typhoid bacilli present in the urine. In such cases, approximately 10 cc. of urine is centrifuged at 3,000 revolutions per minute for 15 minutes and the resulting sediment is cultured

TABLE 1.—Diagnostic Criteria in 41 Cases of Typhoid Fever

Diagnostic Tests	Number Positive	Basis of Diagnosis
Blood culture	6	On blood culture alone. Widal reaction was negative.
Widal reaction	4	On this reaction alone.
Widal reaction and blood culture	20	Based on the two tests alone.
Stool culture and blood culture or Widal reaction (or both)	18	Only in one case was the diagnosis made by fecal culture alone.
Bile culture	5	In one case, there was no other diagnostic response.
Urine culture	2	Both cases also diagnosed by blood culture and Widal reaction.

on *Shigella-Salmonella* and eosin-methylene blue agar and in tryptose broth. Nonlactose fermenters are identified as above.

**Widal Reaction:** The patient's serum is inactivated at 56°C. for 30 minutes. Twofold serial dilutions of serum ranging from 1:40 to 1:5,120 are then made in normal saline solution in 0.5 cc. amounts. Then 0.5 cc. of antigen is added to each tube, giving final dilutions of 1:80 through 1:10,240. A control tube containing 0.5 cc. of saline solution and 0.5 cc. of antigen is included. The tubes are then incubated at 37°C. for 20 minutes, centrifuged at 2,000 r.p.m. for ten minutes, and read for agglutination. The last dilution in each agglutination which occurs is reported as the titer. The antigen is the typhoid "H" antigen and is supplied by the California State Department of Public Health Division of Laboratories.

In regard to the Widal test, it should be remembered that in some cases of typhoid fever, "H" (flagellar) agglutinins do not develop in the blood, while "O" (somatic) agglutinins may be present in relatively high titer. Also, the proportion of cases in which "O" agglutinins develop is much higher (we do not know why) in some communities than in others. Felix and Pitt<sup>1</sup> explained why many virulent strains of typhoid fever do not agglutinate with an "O" antigen: They demonstrated the presence of another special antigen, the "Vi" antigen, as Felix named it. Because the "Vi" antigen is heat-labile, Widal tests cannot detect it unless made with living suspensions of the typhoid organism at 37°C. It is destroyed by being heated to 60°C. for one-half hour, or 100°C. for five minutes. Weak solutions of phenol also destroy it. The presence in a patient's blood of the "Vi" antigen is generally considered *prima facie* evidence of the presence of typhoid fever, whereas the presence of "O" and the "H" antigens may be due to previous vaccination against typhoid fever, to anamnestic reactions or to the Hektoen phenomenon when actually typhoid fever is absent.

The Hektoen phenomenon is one in which a disease other than typhoid fever gives rise to a height-

ened Widal reaction against ordinary typhoid antigens, the specific agglutination being higher, however, against the organism causing the other disease than it is against typhoid fever.

#### DISCUSSION

Of the 41 cases covered by this report, the diagnosis in six was made on the basis of blood culture alone, no Widal reaction being evoked (Table 1). Only four were diagnosed on the basis of the Widal reaction alone. In 20 cases, both tests gave a positive response. Fecal culture was the only positive test finding in a single case; in 18 others, it was confirmed by blood culture or Widal reaction or both. The same was true of aspirated bile culture, which gave the only positive response in one case and was confirmed by other tests in five others. Positive urine culture in two cases was otherwise confirmed in both.

#### NOTES ON TREATMENT

At present, one drug stands out above all others in the treatment of typhoid fever—chloramphenicol. The dosage is most important: In each 24-hour period, 65 mg. per kilogram of body weight for an adult patient and 120 mg. per kilogram for a child. Cures have been reported with smaller doses for children, according to Young's rule, but I have been unable to duplicate these results. In my experience, without the larger dosage for children the disease runs its usual course.

Although chloramphenicol has been incriminated as the cause of malignant neutropenia in some persons, it appears to contribute only rarely to that suppression of hemopoiesis which frequently occurs in typhoid fever and gives rise to leukopenia.

Other drugs have been mentioned in the literature as equally efficient, but the fact that they are neither available nor discussed any longer in the literature makes one doubt their efficacy. At any rate, chloramphenicol is the drug of choice at this moment. It apparently is not bactericidal but bacteriostatic, and one-fourth of all patients in whom the disease

has been brought under control and who have become defervescent by the use of chloramphenicol, will have typhoid fever again at a later date, varying from a few weeks to several months, unless they are vaccinated with typhoid vaccine after they apparently have been cured by chloramphenicol. This drug definitely interferes with the function of immunity.

It has become fashionable to add steroids, particularly cortisone, to the treatment of typhoid fever. Since these drugs also interfere with the mechanism of immunity, it is not wise to employ them routinely in the treatment of typhoid fever when chloramphenicol alone is so efficient.

The temperature and the course of the disease are usually halted within five days after chloramphenicol therapy is begun. However, when this therapy is started late, it will not prevent the usual complications or sequelae, such as hemorrhage or perforation.

At the conclusion of typhoid therapy, because 25 per cent of patients have not developed immunity,

I usually allow patients to remain without treatment for five days to a week and then immunize them with killed typhoid vaccine. From experience, I believe that vaccination is more efficient in the prevention of typhoid recurrences or relapses if the three-dose method of immunization used by the armed services is employed; namely, the use of 500 million killed typhoid organisms for the first vaccine dose, and one billion at each of the two succeeding doses, each given a week apart. At present, there is a tendency to make all three doses the same, each containing only 500 million organisms. The stronger doses, in my opinion, will prevent some of the relapses that still occur while vaccination is being carried out or immediately thereafter.

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#### REFERENCE

1. Felix, A., and Pitt, R. M.: A new antigen of *B. typhosa*. Its relation to virulence and to active and passive immunisation, *Lancet*, 2:186, July 28, 1934.

